Reference: *Biol. Bull.*, **143**: 150–161. (August, 1972)

DIMENSIONS AND ULTRASTRUCTURE OF TOADFISH GILLS

G. M. HUGHES AND I. E. GRAY

Research Unit for Comparative Animal Respiration, The University, Bristol BS8 1UG, England, and Department of Zoology, Duke University, Durham, North Carolina 27706

During the last 20 years the gill areas of many fish species have been measured and a fairly comprehensive impression gained of the overall range among fishes belonging to different groups and having a variety of life habits. It has become clear that more detailed knowledge is now required for individual species and especially in combination with studies on the ultrastructure of the gills and physiology of gas exchange. Of the marine fishes whose gill areas have been measured, the toadfish (*Opsanus tau*) is of particular interest because of the relatively low value found for its gill area (Gray, 1954). This seems to be correlated with the sluggish habits of this species and other aspects of its physiology such as its respiratory dependence down to very low O_2 tension in water (Hall, 1929). This paper is concerned with the fine structure of the toadfish gills together with a more extended analysis of the data summarized previously (Gray, 1954).

Materials and Methods

The toadfish ranged in size from 15–800 g; the gills were removed, fixed, and measurements made according to the method described by Gray (1954). This involved the counting of filaments of the three gill arches, sampling of secondary lamellae and measurement of their areas. The fish were obtained from Woods Hole, Massachusetts, and Beaufort, North Carolina. No significant difference was observed between these two populations. These measurements were analyzed for the relationship between body weight and different components of the gill area using the method of linear logarithmic transformation as described by Muir and Hughes (1969). Regression lines were fitted using the method of least squares with Wang and Olivetti computers. Other toadfish gills were fixed at Beaufort for inspection of their secondary lamellae, and for electron microscopy after fixation in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) with subsequent post-fixation in 1% osmium tetroxide and embedded in Vestopal. The material was examined under AEI 6G and Phillips 200 and 300 electron microscopes.

Results

Gross Morphology

Toadfish gills are reduced relative to those of most fish for there are only three holobranchs on each side. The filaments are widely-spaced along the gill arches and this relative coarseness of the sieve extends to the secondary lamellae as there are only 10–13/mm on each side of a gill filament (Gray, 1954).

A detailed analysis of a single specimen (400 g) showed that the average number of secondary lamellae/mm was 11.0, 12.7 and 11.6 for the tip, middle and

base of a filament. The filaments of the posterior hemibranch are longer than the anterior filaments for the first and second arches, but the anterior filaments are longer on the third arch (Fig. 1).

Total gill area and body weight. The relationship between total gill area and body weight for 58 specimens that had been measured, is plotted on log/log coordinates in Figure 2. It is clear that the gill area increases with increasing size, the regression line obeying the equation

$$\text{Log } A = 560.7 + 0.79 \log W$$
$$(i.e., A = 560.7\ W^{0.79})$$

Correspondingly the regression line for gill area/g against body weight has a slope of -0.217. A summary of the gill area data divided into 50 g classes is given in Table 1. The slope of the regression line relating these average areas to body weight is 0.779.

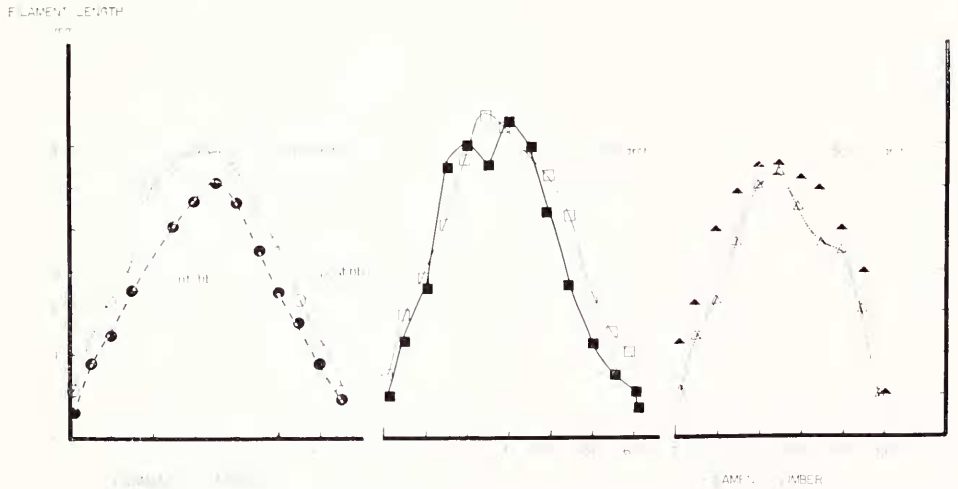


FIGURE 1. Graphs to show the length of filaments on the different gill arches of the left side of a toadfish (*ca.* 400 g). Solid symbols indicate the lengths of filaments of the anterior hemibranches in each case. On average, every 5th filament was measured.

Components of the gill area. When the measurements for gill area determinations are examined, it is clear that the increased area of larger fish is mainly due to a greater total number of secondary lamellae (Fig. 3 C). The number of gill filaments increases rapidly at body weights up to 50 g, but above that size there is relatively little increase in the total number of filaments (N). Filament length increases continuously and consequently the number of secondary lamellae (Fig. 3). This is apparent when the data are plotted on log/log coordinates showing that the regression line for an increase in number of secondary lamellae has a slope of 0.42 whereas that for filament number increases as $W^{0.087}$. Another important factor is the increased area of the secondary lamellae themselves, as indicated below.

The secondary lamellae are relatively large in the toadfish, and as in other species their shape varies according to their position on the gill arches and espe-

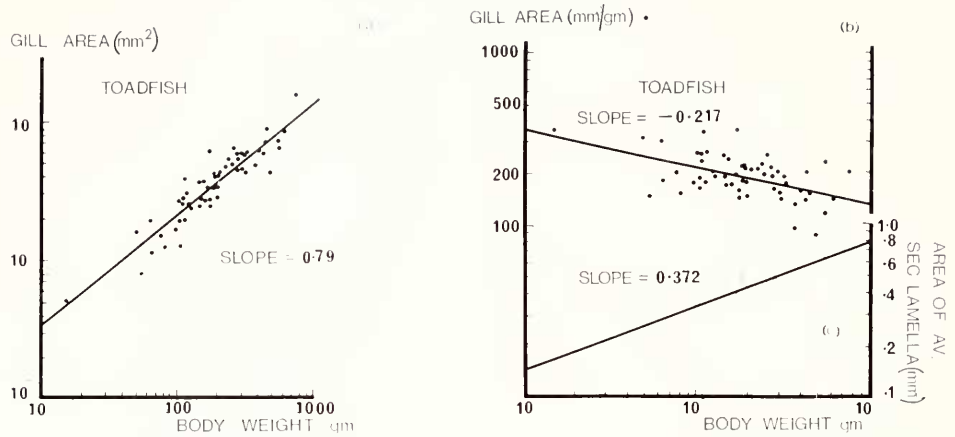


FIGURE 2. Relationships between areas of the gill system and body weight plotted on log/log coordinates: (a) relationship between total gill area and body weight; (b) gill area/g and body weight; (c) the unweighted average area of a secondary lamella and body weight.

cially on the gill filament (Fig. 4). Variations in shape and area of the secondary lamellae from different parts of the gill are related to the flows of water and blood and their role in gas exchange, about which little is known for toadfish. Secondary lamella areas can be plotted out in different ways, indicating their increasing area in the direction of water flow (Fig. 4). Such ways of summarizing the form of

TABLE I

Average weights and gill areas for 50 g classes of 58 specimens of toadfish. Averages for fish within the same order of magnitude are also given

Wt. class	Average wt.		Gill area	
	g	No.	mm²/fish	mm²/g
0–50 g	15	(1)	5,236	349.07
50–100	69.5	(7)	14,199	204.3
100–150	120.4	(13)	26,217	217.75
150–200	182	(13)	35,248	193.67
200–250	232	(4)	48,737	210.07
250–300	277.6	(5)	56,906	204.99
300–350	317.5	(4)	52,652	165.83
350–400	374	(2)	42,168	112.75
400–450	425	(3)	71,529	168.30
450–500	471	(2)	56,977	120.97
500–550		(0)		
550–600	560	(2)	69,207	123.58
600–650	620	(1)	86,867	140.11
650–750		(0)		
750–800	776	(1)	160,362	206.65
10–100	62.7	(8)		228.75
100–1000	260	(50)		191.46

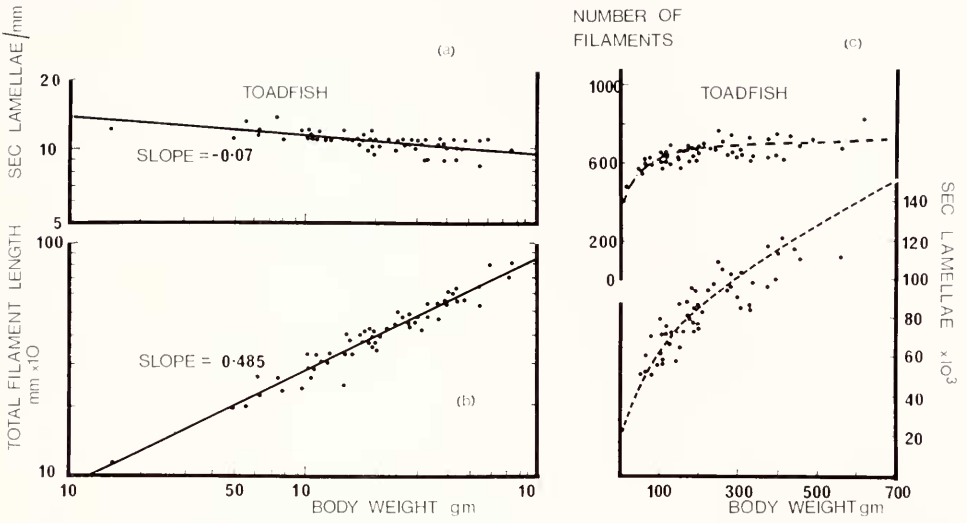


FIGURE 3. Graphs to show (a) relationship between average number of secondary lamella/mm on one side of a filament and body weight plotted on log/log coordinates; (b) log/log plot of the total filament length against body weight and (c) the increase in total number of gill filaments and secondary lamellae (dotted line) of 58 toadfish.

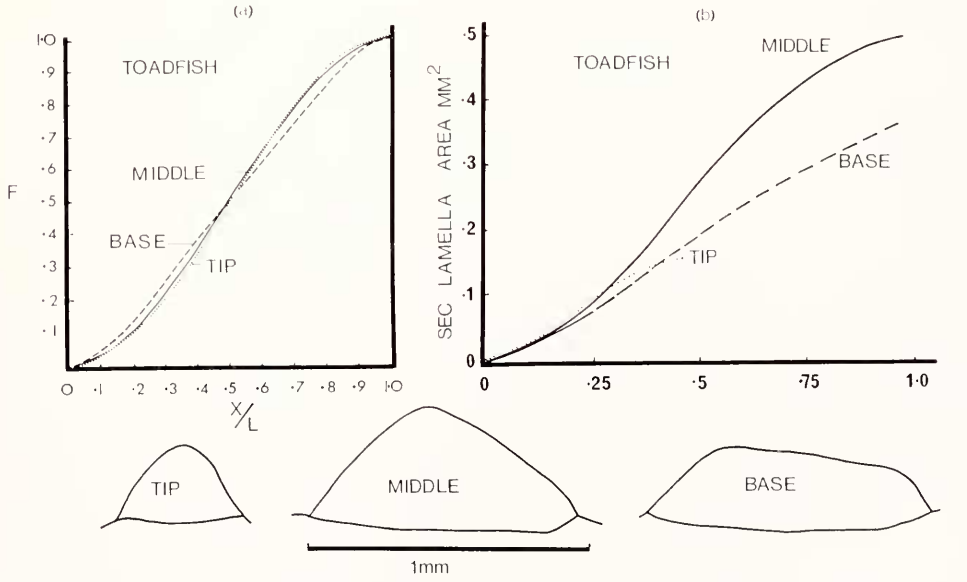


FIGURE 4. Outline shapes of the secondary lamellae from the tip, middle and base of filament 45 of arch 2 of a 400 g toadfish. The change in area of the secondary lamellae in the direction of water flow are plotted (a) as fractional cumulative areas (F) with respect to the fractional path length (X/L), (b) as cumulative areas (mm^2) along the length of the secondary lamella in the direction of water flow.

TABLE II

Opsanus tau. *Results of regression analysis for the gill area and its component parameters.*

	W = 1 g			W = 10 g		
		Limits			Limits	
		95% Conf	Tol		95% Conf	Tol
Total fil length (mm)	301.2	363.3 250.9	394.4 231.4	923.7	1028 830.7	1150 741.8
No. sec. lam./mm on one side of filament	15.86	18.33 13.71	19.53 12.87	13.37	14.53 12.30	15.87 11.27
Ave. area of sec. lam. (mm^2)	0.06037	0.09781 0.03726	0.1206 0.03021	0.1412	0.1866 0.1082	0.2502 0.08074
Total area (mm^2)	560.7	921.9 341.0	1144 274.9	3459	4599 2604	6024 1929
Wt. specific area (mm^2 g)	585.6	934.2 367.1	1050 326.6	355.4	462.9 272.9	551.2 229.2

a secondary lamella are of value in analyses of the O_2 tension gradients along the secondary lamella (Hughes and Hills, 1971; Hughes, 1972a, 1972b). In Gray's (1954) original data, secondary lamellae were termed "lamellae" or "platelets" and refer to the *two* secondary lamellae which are more or less opposite at a given level on a gill filament. Hence for comparison with data for other species it is usually necessary to halve his figures for areas of individual secondary lamellae and to double the total numbers of secondary lamellae.

The increase in total number of secondary lamellae with body weight is largely due to the increased length of the gill filaments, for the spacing (1/d') remains relatively constant. The data for total filament length and number of secondary lamellae/mm are plotted on log/log coordinates in Figure 3 A and B and the relevant statistical information is given in Table II. Thus:

Secondary lamellae/mm on one side of filament = $15.86\ W^{0.074}$; and
Total filament length (mm) = $302.1\ W^{0.485}$

Fine structure of the toadfish secondary lamella

The structure of the secondary lamella as seen under the electron microscope is similar to that of other teleost fish (Fig. 5) (Hughes and Grimstone, 1965; Newstead, 1967; Hughes and Wright, 1970; Tovell, Morgan and Hughes, 1970). The outer epithelial layers are separated from the pillar cell flanges by a well-marked basement membrane which consists of three well-defined layers: (i) an outer clear layer, (ii) a fine fibrous layer, followed on the inner side by (iii) a much thicker collagenous layer being about 4 or 5 times the thickness of the other two layers, which together constitute the basal lamina (Figs. 6, 8). In some places the outer homogeneous layer seems to have protuberances into the inner border of the epithelial layers. The collagen fibrils have clearly defined striations,

TABLE II

Values for 1, 10, 100, and 1000 g fish are given, together with the confidence and tolerance limits

W = 100 g			W = 1000 g			Y = aW^b			
	Limits			Limits					
	95% Conf	Tol		95% Conf	Tol	b	S_b	a	S_a
2825	2920 2732	3433 2325	8638	9191 8117	10570 7060	0.4854	0.0174	302.1	1.097
11.27	11.51 10.98	13.12 9.69	9.51	9.98 0.96	11.13 8.13	−0.0740	0.0137	15.86	1.075
0.3347	0.3624 0.3090	0.5529 0.2026	0.7879	0.9301 0.6676	1.1329 0.4672	0.372	0.046	0.0604	1.274
21350	23330 19530	35850 12720	131700	156500 110900	225800 76850	0.790	0.047	560.7	1.282
215.7	230.1 202.1	308.0 151.0	130.9	152.4 112.4	191.8 89.32	−0.217	0.045	585.6	1.265

repeating every 640 Å. The collagen layer is particularly thick next to each pillar cell body where it gives rise to the columns.

Because of its thick collagen layer, the toadfish gill is especially suitable for inspection of the structure of the columns which, as in other species, are extracellular. The number of columns/pillar cell is about five. The not uncommon folding observed in the pillar cell columns (Fig. 6) perhaps suggests a contracted condition of these cells at fixation. In both transverse and longitudinal sections of the pillar cells, it is apparent that the columns also contain a type of fine fibril which is not, however, a direct continuation of the fine fibrous layer of the basal lamina (Fig. 6). In the pillar cell flanges, fibrils appear in cross-section which are very suggestive of collagen. Another interesting feature observed in toadfish pillar cells is the presence of cytoplasmic processes jutting into the blood channel from the main cell body or its flanged part. These processes sometimes contain what appear to be collagenous fibrils. The pillar cell body contains many types of granule and is well provided with cytoplasmic filaments suggestive of contractile protein, particularly in the neighborhood of the columns. There is also some evidence of such filaments in the endothelial cells which line the marginal channel of the secondary lamella. Typical endothelial granules (Weibel and Palade, 1964; Hughes and Wright, 1970; Weibel and Hughes, in preparation) were specially prominent in the toadfish and their presence in these cells only and not in the pillar cell flanges was very clearly defined in most cases. In addition, the endothelial cells have many pinocytotic vesicles and specially large ones are often seen bordering the collagen layer of the basement membrane. Unlike the comparable layer of the mammalian lung, this endothelium has no underlying basal lamina.

The outer epithelial layers have a number of points of interest. Microvilli are not very obvious in most sections but there seems to be some surface sculpturing as there are deep invaginations between epithelial cells, especially in the crypts

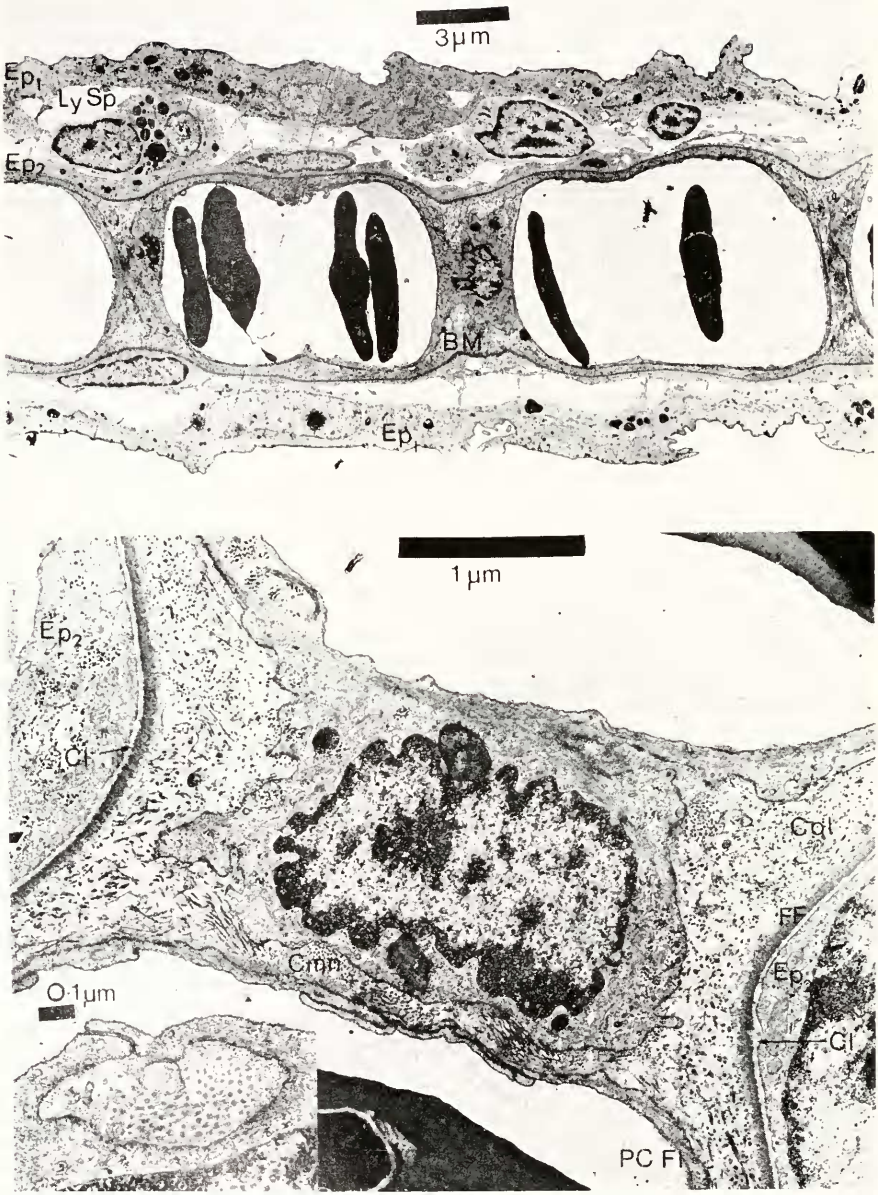


FIGURE 5. *Opsanus tau*. Electron micrograph to show the basic structure of a secondary lamella cut transverse to the direction of blood flow. Three pillar cells (PC) are visible separating and lining the blood channels. Notice the sculpturing of the outer epithelial layer (Ep_1) and the different types of lymphocyte (monocytes and macrophages) to be found in the lymphoid space (Ly Sp), between the two epithelial layers. The basement membrane (BM) separates the inner epithelial layer (Ep_2) from the pillar cells.

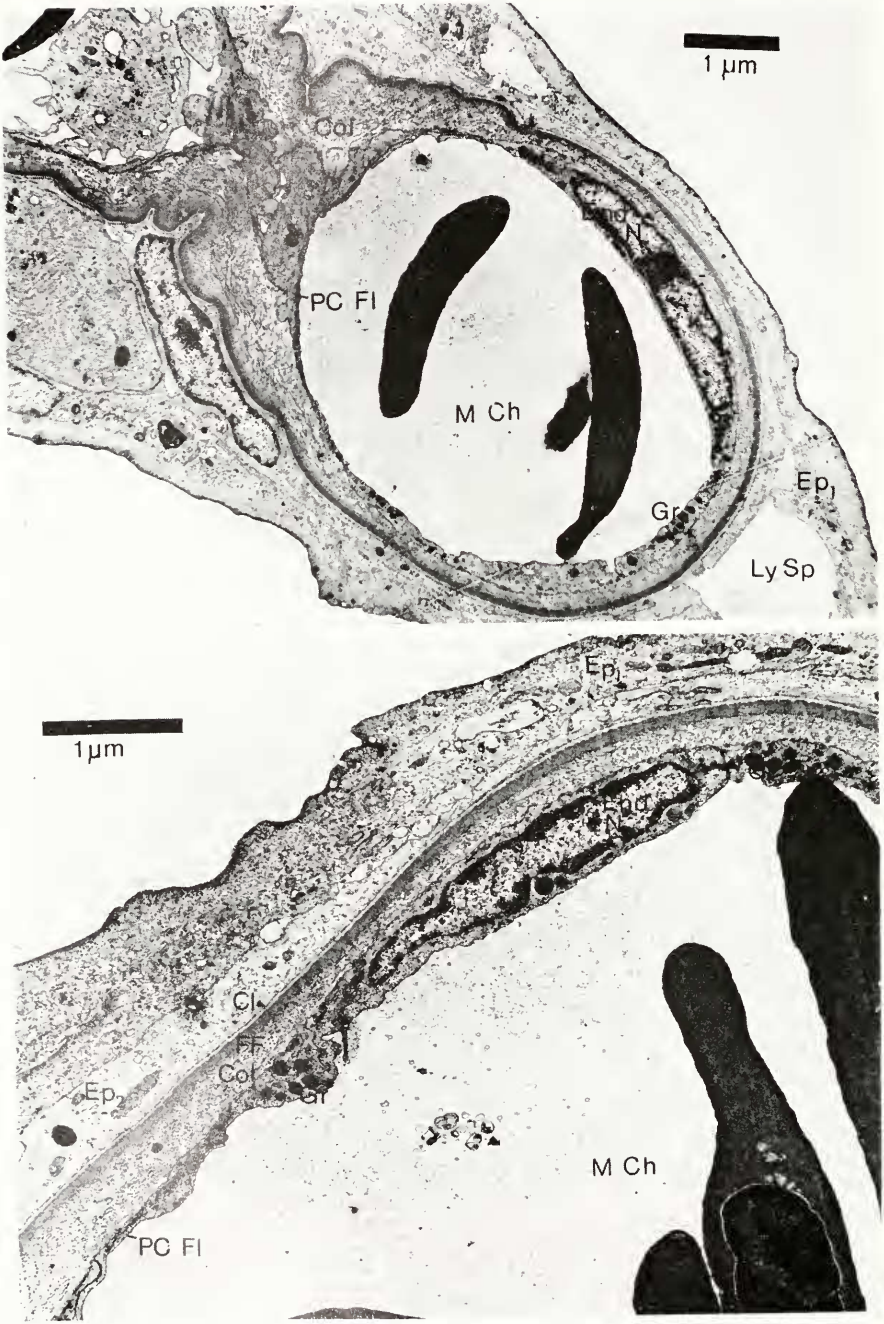
between secondary lamellae. Many fine-folded junctions are found between cells of the outer epithelial layer and where these come to the surface they sometimes resemble microvilli similar to those noticed in the pollack between adjacent epithelial cells. Desmosomes are also visible at these junctions. Epithelial cells in the region outside the marginal channel are often particularly thick (Fig. 7). Frequently the outer layer of epithelial cells seems to form a continuous coat which may be separate to some extent from the underlying epithelial cells. These "lymphoid" spaces often contain a number of lymphocytes of different types but the space clearly does not contain plasma. Transitional stages from monocytes to macrophages are often visible (Fig. 5). Amoebocytes are also present in the blood channels (Fig. 7), but no connection has been established between these channels and the intra-epithelial spaces. The outer surface of the epithelial cells is more darkly staining than the rest. Chloride cells are common, both in the crypts and other regions of the secondary lamella and often seem to be separated from the outer surface by the outer epithelial layer. The cytoplasm of the chloride cells is also somewhat unusual.

The water/blood pathway is relatively thick in certain parts of the sections, but in others it may be as thin as 3 μm. The epithelial layers usually constitute from $\frac{1}{2}$ to $\frac{3}{4}$ of the total water/blood distance. The flange and endothelial layers are very thin and, as mentioned above, the basement membrane and particularly its collagen layer, is noticeably thickened in this species. The collagen layer is at least 1 μm thick.

Discussion

The toadfish data analyzed in this paper are more extensive than that available so far for adults of any other marine species. The results obtained are in substantial agreement with those given for tunas (Muir and Hughes, 1969) with which they contrast considerably because of the great differences in activity of the two species. Some of the most obvious differences in gill dimensions are shown in Table III which summarizes the "a" and "b" values in the relationship $Y = aW^b$, where Y is the gill area or its constituent parameters. Clearly the slope of the log/log regression line for total gill area of tunas is greater than that of toadfish and similar differences are seen in the component parameters; for both fish they are all of the same order of magnitude. Far greater differences are observed in the "a" or intercept values, *e.g.*, that for total area of tuna is 6 times that of toadfish. This is made up of a 16-fold difference in the figure for total filament length and a four-times greater number of secondary lamellae/mm. However, for the area of an average secondary lamella the "a" value for toadfish is about 14 times greater than for tuna. These differences clearly support the generalization that more active fish tend to have a greater number of closely-spaced secondary lamellae which are of relatively smaller area (Hughes, 1966). Figures for the dolphin

Figure 6. A single pillar cell from a secondary lamella of the toadfish showing its flange (PC Fl) lining the blood spaces, the thickened collagen layer (Col) of the basement membrane, and a single column (Cmn) in longitudinal section. The fine fibrous (FF) and outer clear (Cl arrow) layers of the basal lamina can easily be distinguished. A transverse section across a single column is shown at higher magnification as an insert, bottom left.



FIGURES 7–8.

TABLE III

Comparison of the intercept (a) and slope (b) of the regression lines for the components of the gill areas of toadfish, Coryphaena *and tunas**

		Toadfish		Coryphaena		Tunas	
		a	b	a	b	a	b
Total filament length (mm)	L**	302.1	0.485	1879	0.431	5594	0.382
Secondary lamellae/mm on one side of filament	$\frac{1}{d'}$	15.96	−0.079	33.81	−0.036	60.87	−0.089
Average area of sec. lam. (mm^2)	bl	0.0604	0.372	0.0377	0.327	0.0046	0.583
Total area (mm^2)	A	560.7	0.79	5208	0.713	3151	0.875

* Based on Muir and Hughes (1969).
** Symbols as in Hughes (1966).

fish, *Coryphaena* (Table III) are closer to tuna, and data for other fish fall between the toadfish and *Coryphaena*.

From the respiratory point of view, a very important parameter is the distance (t) separating the blood and water which is substantially greater in the toadfish than in the tuna. The general relationship between O_2 consumption ($\dot{V}_{O_2}$) and area of the respiratory surfaces (A) is given in the equation:

$$\dot{V}_{O_2} = \frac{KA\ \Delta P_{O_2}}{t}$$

which may be rearranged:

$$\frac{\dot{V}_{O_2}}{\Delta P_{O_2}} = K \cdot \frac{A}{t} = D_t,$$

the diffusing capacity of the tissue barrier of the gills (Hughes, 1972b). K is the permeation constant of Krogh expressed as ml $O_2/\mu m/cm^2/mm$ Hg and is usually assumed to be the same for the different layers of the water/blood barrier which has an overall thickness *t*. However, if there are marked differences in K between these layers then the much greater thickness of the collagen layer in toadfish could be significant.

The available figures give estimates for gill area of a typical 100 g toadfish of about 21,000 mm^2 whereas that of a bluefin tuna of the same size would be of the order of 200,000 mm^2. The water/blood distances are approximately 5 μm for

FIGURE 7. Section through the marginal channel of a toadfish secondary lamella. The nucleus (End N) of an endothelial cell is clearly visible but the section only passes through the edge of a pillar cell of the outer row. Bordering the latter a greatly thickened collagen layer (Col) can be seen. The epithelial layers along the outer edge of the marginal channel (M Ch) are thickened and separated by lymphoid spaces (Ly Sp), so that the water/blood distance is much greater here than on the lateral aspects of this channel.

FIGURE 8. Higher magnification electronmicrograph of a part of the marginal channel (M Ch) to show the different components of the water/blood barrier. The section passes through a flattened endothelial nucleus (End N) and typical granules (Gr) are visible in this cell which clearly differentiate its cytoplasm from that of the pillar cell flanges (PC Fl) which line most of the blood channels.

toadfish and 0.5 μm in tunas (Hughes, 1970). Consequently the diffusing capacities (D_t) are about 42 K and 4000 K, respectively. Thus the same differences in O_2 tension across the gills would result in the transfer of an amount of O_2 that is about 100 times greater for the tuna than a toadfish. This example clearly emphasizes the importance of the differences in area and thickness of the two species, but as yet no physiological measurements have been made of the diffusing capacity of the gills (D_g) in these two species so that these estimates must remain anatomically-based.

Ultrastructural studies of the toadfish gill have also served to emphasize one or two interesting features which are probably general for most fish gills.

As mentioned previously, the collagen layer of the basement membrane is particularly well developed in this fish and emphasizes its importance as a supporting structure in these relatively coarse gills, which may continue to function when the fish is out of water. Two epithelial layers are present as in most other species, but in the toadfish the space observed between these two layers is particularly noticeable. The presence of macrophages and other leucocytes in the lymphoid space suggests a protective function, perhaps analogous to the alveolar macrophage of the mammalian lung. Clearly the respiratory surface of the fish being constantly ventilated by water could not have macrophages on its outer surface. Thus the presence of such cells between the two epithelial layers can be related to the difference in respiratory medium. This space has generally been omitted in discussions of the water/blood barrier of fish. If it is taken into account, the constituent layers can be listed as follows (1) outer epithelial layer; (2) lymphoid space; (3) inner epithelial layer; (4) basal lamina; (5) collagen layer; (6) pillar cell flange—instead of epithelium (1, 2, 3), basement membrane (4 and 5) and pillar cell flange (6).

Comparison of the fine structure of the toadfish secondary lamella with that of tuna is also instructive and emphasizes the correlation between structure of the respiratory surface and the habits of the animals.

We wish to thank Knut Schmidt-Nielsen and Vance Tucker for providing and assisting with computer facilities. Analysis of this data was begun during a visit of G. M. H. to the Department of Zoology at Duke University, N. Carolina, and both there and at Beaufort he enjoyed excellent hospitality.

Summary

1. This analysis of the measurement from the gills of about 60 toadfish using log/log transformation has shown that the gill area and its constituent parts increases with body weight as follows:

Total area (mm^2) = $560.7\ W^{0.79}$
Total filament length (mm) = $302.1\ W^{0.485}$
Secondary lamellae/mm on one side of filament = $15.99\ W^{-0.075}$
Average bilateral area of a secondary lamella (mm^2) = $0.06\ W^{0.372}$

2. A comparison with the corresponding data for other fish, particularly tunas, shows differences in the values of these relationships and confirms the general

conclusion that the gills of more sluggish fish have a smaller number of larger secondary lamellae and relatively wider spaces through which the water flows.

3. An electron microscope study of the toadfish secondary lamella has shown the same basic structure as in other teleost fish but the collagen layer of the basement membrane is especially thick. Also noticeable are distinct lymphoid spaces between the two epithelial layers and the presence of stages in the development of cells concerned with the protection of these layers. The water/blood barrier therefore comprises the following layers in certain regions: (1) outer epithelial layer, (2) lymphoid spaces, (3) inner epithelial layer, (4) basal lamina, composed of outer clear and inner fine fibrous layers, (5) collagen layer, (6) pillar cell flange.

LITERATURE CITED

Gray, I. E., 1954. Comparative study of the gill areas of marine fishes. *Biol. Bull.*, **107**: 219–225.

Hall, F. G., 1929. The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Amer. J. Physiol.*, **88**: 212–218.

Hughes, G. M., 1966. The dimensions of fish gills in relation to their function. *J. Exp. Biol.*, **45**: 177–295.

Hughes, G. M., 1970. Morphological measurements on the gills of fishes in relation to their respiratory function. *Folia Morphologica*, **18**: 78–93.

Hughes, G. M., 1972a. Distribution of oxygen tension in blood and water along the secondary lamella of icefish gills. *J. Exp. Biol.*, **56**: 481–492.

Hughes, G. M., 1972b. Morphometrics of fish gills. *Respir. Physiol.*, **14**: 1–26.

Hughes, G. M., and A. V. Grimstone, 1965. The fine structure of the secondary lamellae of the gills of *Gadus pollachius*. *Quart. J. Microscop. Sci.*, **106**: 343–353.

Hughes, G. M., and B. A. Hills, 1971. Oxygen tension distribution in water and blood at the secondary lamella of the dogfish gill. *J. Exp. Biol.*, **55**: 399–408.

Hughes, G. M., and D. E. Wright, 1970. A comparative study of the ultrastructure of the water/blood pathway in the secondary lamellae of teleost and elasmobranch fishes—benthic forms. *Z. Zellforsch. Mikrosk. Anat.*, **104**: 478–493.

Muir, B. S., and G. M. Hughes, 1969. Gill dimensions for three species of tunny. *J. Exp. Biol.*, **51**: 271–285.

Newstead, J. D., 1967. Fine structure of respiratory lamellae of teleostean gills. *Z. Zellforsch. Mikrosk. Anat.*, **79**: 396–428.

Tovell, P. W., Miriam Morgan and G. M. Hughes, 1970. Ultrastructure of trout gills. *17th Congrès Internat. de Microscopie Electronique*, Grenoble, **3**: 601.

Weibel, E. R., and G. E. Palade, 1964. New cytoplasmic components in arterial endothelia. *J. Cell Biol.*, **23**: 191–212.